Yarrowia lipolytica LithAc transformation v1.0

Introduction

Transformation protocol from CAHOL, who works at CfB. Is optimized by .

Materials

- MilliQ water
- YPD plates
- Appropriate selection reagents
- YPD media
- Micro centrifuge
- 1.5mL eppendorf tubes
- Either:
  - Bürger-Türk hemocytometer with depth 0.1 mm squares of size C (0.2 mm x 0.2 mm)
  - Microscope
- Or:
  - Cuvette
  - OD meter
- Transformation mix
  - Polyethylene glycol (PEG)
  - Lithium Acetate (LiAc)
  - Salmon sperm DNA (ssDNA)
  - Dithiothreitol (DTT)

Procedure

Prepare stock solutions for transformation mix

1. Prepare stock solutions using this protocol: http://openwetware.org/wiki/Springer_Lab:_TransformationYeast
2. Mix to prepare transformation mix

   Make sure that all DTT is resuspended; it might be hard to see, but is crucial for good transformation efficiencies

Table1

	A	B	C	D
1		1x (µL)	10x (µL)	12x (µL)
2	PEG (Stock 50%; sterile-filtrated; end 43.8%)	87.5	875	1050
3	LiAc (Stock 2M; sterile-filtrated; end 0.1 M)	5	50	60
4	ssDNA (Stock 10 mg/ml; end 0.25 g/l)	2.5	25	30
5	DTT (stock 2M; sterile-filtrated; end 100 mM)	5	50	60
6	**Total**	**100**	**1000**	**1200**

Day 1

3. Plate colony resuspended in 100 microliter MilliQ on YPD plates
4. Incubate plates for 24h @ 30°C

Day 2

5. Softly resuspended entire plate of cells in 1 mL MilliQ. Wash twice.
6. Centrifuge at 3000 RPM for 5-10 min.
7. Dilute cells 100-200x in MilliQ
8. Count using a Bürger-Türk hemocytometer with depth 0.1 mm. Count cells in 8 squares of size C (0.2 mm x 0.2 mm).
9. Calculate cells/mL = avarage of cells counted in 8 squares/volume of i square in mm^3 * dilution factor x 10^3
10. Otherwise: measure OD_{600} and use a final OD of 9.2 per transformation
11. For each transformation use 5 x 10^7 cells resuspended in 100µL transformation mix and 500ng DNA (max 5µL)
12. Incubate @39°C with shaking ~200RPM for 1h

01:00:00

13. If no antibiotics are used, plate the transformation mix directly on selective plates
14. If antibiotics are used, centrifuge the culture for 10 min at 3000 rpm and recovered in 600µL YPD for 2h before plating on selective plates.

02:00:00

15. Incubate @ 30°C for 2 days.

48:00:00